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Targeting one-carbon metabolism by methyl donors for prevention of non-alcoholic fatty liver in rats

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Abstract

Aim: The purpose of the current work was to demonstrate and compare the impacts of folate, methionine, and choline as methyl donors in the prevention of NAFLD induced by a high-fructose diet (HFrD).

Material and Methods: Sixty adult male albino rats were divided into five groups: G1 had a control-fed basal diet; in G2, the rats were fed an HFrD containing 45% fructose (HFrD); in G3, G4, and G5, the rats were fed an HFrD supplemented with folic acid, L-methionine, and choline chloride, respectively. The present study's findings showed a close connection between methyl donors and lipid metabolism.

Results: The findings indicated that the folic acid supplemented diet (HFrD+ folate) exhibited the most substantial improvement in hepatic total lipids (TP), triacylglycerol (TG), phospholipid (PL), serum total cholesterol (TC), low-density lipoprotein cholesterol (LDL-c), free fatty acid (FFA), albumin (ALB), total bile acid (TBA), fasting blood glucose (FBG), proinflammatory cytokines levels, and enzyme activities of alanine transaminase (ALT), aspartate transaminase (AST), fatty acid synthase (FAS), and acetyl coenzyme A carboxylase (ACC). In the same context, L-methionine had the most significant improvement in oxidative stress biomarker (glutathione [GSH], superoxide dismutase [SOD], 4-hydroxynonenal [4-HNE]) levels, followed by folic acid and choline.

Discussion: Methyl donors such as folic acid, methionine, and choline are essential for maintaining OCM and can prevent animals with NAFLD from developing liver lipid accumulation.

Keywords

One-Carbon Metabolism, L-Methionine, Choline, Folic Acid, Methyl Donors

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Introduction

Nonalcoholic fatty liver disease (NAFLD) is a combination of conditions that are caused by an excessive buildup of fat in the liver. However, no medications have been approved for the treatment of NASH [1]. Therefore, since NAFLD is now considered a significant global health burden, discovering new drug targets for the treatment of NASH is vitally important.

A metabolic mechanism called one-carbon metabolism (OCM) recycles one carbon atom through the methionine and folate cycles. This metabolism is vital for the management of amino acids, glucose, and vitamins in addition to the biosynthesis of macromolecules for the conservation of oxidation-reduction equilibrium and for the stabilization of the reaction of the methyl group, which makes methyl-group metabolism the link between epigenetic control and intermediate metabolism [2]. Various biochemical molecules such as vitamins and amino acids fuel the OCM cycle. As well as, the by-products of OCM are vital for a number of cellular functions.

Folic acid acts as a carbon donor in the production of purines/pyrimidine bases and serine from glycine. In order to re-methylate homocysteine into methionine, it also serves as a methyl donor to produce methylcobalamin. It has been demonstrated that dietary folic acid prevents the accumulation of hepatic lipids [3]. Folic acid and other methyl donors have been shown in numerous studies to have beneficial effects on chronic liver diseases [4].

Methionine is an essential amino acid for protein synthesis and growth, and free methionine is absorbed and converted to S-adenosylmethionine (SAM) [5]. SAM acts as a main sulfate and methyl-group donor in various biochemical reactions and is recommended for the treatment of particular diseases. Methionine metabolism, one of the key one-carbon metabolic pathways, connects the transsulfuration pathway to the folate cycle. It also acts as a precursor for the production of glutathione (GSH) in addition to being the main methyl donor for the methylation of proteins, histone, phospholipids, nucleic acids, and biogenic amines [6].

Choline, a main methyl donor, is a crucial nutrient and a fundamental component of various essential neurochemical and physiological reactions. Choline deprivation boosts the reduction of free radicals from mitochondria due to changed mitochondrial membrane structure and accelerated fatty acid oxidation. An absence of choline also changes DNA methylation and decreases folate metabolism and thymidylate production [7]. The aim of the study is to assess the impacts of folate, methionine, and choline as methyl donors in the prevention of NAFLD induced by a high-fructose diet (HFrD).

Material and Methods

Chemicals

Folic acid, L-methionine, and choline chloride were purchased from Now Foods company for natural food supplements, Bloomingdale, IL, USA.

Animals and Experimental Design

Adult male albino rats (8–10 weeks old) weighing between 128–137 g were used in the current study. The study was conducted from December 2022 to March 2023 and was approved by the Animal Care and Use Ethics committee at King Fahd Medical Research Center (KFMRC), King Abdulaziz University, Jeddah, Saudi Arabia. The rats were kept in air-conditioned animal housing at a temperature of 24°C in stainless steel cages, fed basal diet, and permitted water ad libitum throughout the experimental period (10 weeks). Sixty rats were classified into five groups (12 rats/group) as follows:

[1] Group 1 (Control): Healthy rats fed a basal diet. The control rats received an isocaloric diet (protein 18.3%, starch 66.5%, and fat 5.2%).

[2] Group 2 (HFrD): Rats fed an HFrD (45 g fructose/100 g diet) to induce NAFL. The rats received a diet containing 18.3% protein, 66.5% carbohydrate (consisting of 45% fructose and 21.5% starch) and 5.2% fat.

[3] Group 3 (HFrD + folic acid): Rats fed a diet of HFrD supplemented with 20 mg of folic acid/100 g.

[4] Group 4 (HFrD + met): Rats fed HFrD supplemented with 0.9 g L-methionine/100 g.

[5]: Group 5 (HFrD + chol): Rats fed HFrD supplemented with 0.6 g choline chloride/100 g.

Sample Collection and Biochemical Assessment

After 10 weeks, blood was drawn from the hepatic portal vein of ether-anesthetized rats that had fasted overnight. Serum samples were kept frozen at –20ºC for later biochemical testing. The livers were removed, washed out with saline solution (NaCl 0.9%), then blotted on filter paper, weighted, and frozen immediately at –20ºC for subsequent analysis. Liver lipids were extracted using the chloroform-methanol extraction technique. Kits of total lipids (TL), total cholesterol (TC), triacylglycerols (TG), total phospholipids (PL), high-density lipoprotein cholesterol (HDL), alanine transaminase (ALT), aspartate transaminase (AST), albumin (ALB), and total bile acids (TBA) were purchased from Siemens Healthcare Diagnostics, U.S.A. Kits for assessing fatty acid synthase (FAS), acetyl coenzyme A carboxylase (ACC), and free fatty acids (FFA) were purchased from Bioassay Technology Laboratory (BT LAB), UK. Reduced GSH, 4-hydroxynonenal (4-HNE), and superoxide dismutase (SOD) kits were purchased from Bio Vision Inc. Co., USA. Kits for the assessment of tumor necrosis factor (TNF-α), interleukine-6 (IL-6), and interleukine-1 β (IL-1β) were purchased from Kamiya Biomedical Co. CA. USA.

Statistical Analysis

Data were statistically analyzed using SPSS software program (version 22.0) The results were presented as mean ± standard error (n = 12). The one-way analysis of variance (ANOVA) test was used to find the differences between mean values. Statistics were considered significant for P values under 0.01.

Ethical Approval

Ethics Committee approval for the study was obtained.

Results

HFrD rats showed a significant increase ($P \leq 0.01$) in absolute and relative liver weight compared with the control group. However, those that consumed folate and choline supplemented diets showed a significant reduction ($P \leq 0.01$) in both relative and absolute liver weights (Table 1).

Rats in the HFrD group showed significant dyslipidemia. Hepatic TG and TL and serum TC and LDL values were significantly elevated compared with the control rats, with decreased serum

Table 1. The effect of various treatments on absolute and relative liver weight.

Groups	Absolute liver weight (g)	Relative liver weight (g)
Control	7.21 ± 1.10	3.27 ± 0.35
HFrD	9.85 [a] ± 1.78	4.95 [a] ± 0.62
HFrD + folate	7.76 [b] ± 0.57	3.84 [b] ± 0.25
HFrD + methionine	8.54 [a] ± 0.70	4.24 [a] ± 0.35
HFrD + choline	7.33 [b] ± 0.52	3.94 [b] ± 0.15

Values are expressed as means ± SE. (n = 12); a = significance against control; b = significance against HFrD group; c = significance against HFrD+ folate group; d = significance against HFrD+ Met. at P ≤ 0.01

Table 2. The effect of experimental diets on the levels of biochemical variables.

Groups	Control	HFrD	HFrD + folate	HFrD + met.	HFrD + chol.
TL (mg/g)	62.85 ± 3.20	145.5 [a] ± 7.5	71.0 [ab] ± 5.5	122.2 [abc] ± 8.1	86.2 [abcd] ± 5.3
TG (mg/g)	59.51 ± 2.30	101.2 [a] ± 4.5	63.8 [b] ± 2.3	92.5 [ac] ± 4.1	87.5 [abc] ± 3.8
Total PL (mg/g)	23.21 ± 1.90	16.7 [a] ± 1.3	22.2 [b] ± 2.1	18.7 [ac] ± 1.1	21.2 [bd] ± 2.3
FBG (mmol/L)	4.150 ± 1.08	8.95 [a] ± 1.25	5.55 [ab] ± 1.02	8.05 [abc] ± 1.11	6.22 [abd] ± 1.21
TC (mg/dl)	143.50 ± 1.3	235.1 [a] ± 9.1	152.3 [ab] ± 3.5	170.1 [abc] ± 5.2	172.3 [abc] ± 6.1
HDL-c (mg/dl)	65.20 ± 1.05	32.7 [a] ± 0.80	59.1 [ab] ±0.90	55.2 [abc] ± 0.50	56.2 [abc] ± 0.30
LDL-c (mg/dl)	52.40 ± 0.05	188.3 [a] ± 4.10	69.8 [ab] ± 1.10	93.4 [abc] ± 1.06	87.5 [abc] ± 0.05
ALB (g/dl)	4.60 ± 0.30	2.55 [a] ± 0.2	3.90 [b] ± 0.4	2.85 [ac] ± 0.3	3.55 [abd] ± 0.5
TBA (µmol/L)	36.30 ± 2.3	73.5 [a] ± 2.5	39.1 [ab] ± 1.9	69.5 [abc] ± 2.5	42.5 [abd] ± 2.5
ALT (IU/L)	42.50 ±1.80	142.5 [a] ± 7.5	67.2 [ab] ± 4.1	100.3 [abc] ± 4.9	78.5 abd ± 4.8
AST (U/L)	69.30 ± 4.20	222.3 [a] ± 8.1	79.3 [ab] ± 5.3	185.3 [abc] ± 9.7	109.1 [abcd] ± 3.9
FFA (µmol/ml)	0.650 ± 0.07	1.50 [a] ± 0.06	0.85 [ab] ± 0.08	1.05 [ac] ± 0.04	0.99 [ab] ± 0.06
FAS (ng/ml)	35.55 ± 2.10	72.7 [a] ± 3.10	45.22 [ab] ± 2.2	58.13 [abc] ± 3.2	56.05 [abc] ± 2.8
ACC (ng/ml)	7.05 ± 0.055	11.52 [a] ± 1.00	8.02 [ab] ± 0.05	8.95 [ab] ± 0.09	8.80 [ab] ± 1.00

Values are expressed as means ± SE. (n = 12); a = significance against control; b = significance against HFrD group; c = significance against HFrD+ folate group; d = significance against HFrD+ Met. at P ≤ 0.01

Table 3. The effect of experimental diets on oxidative stress biomarkers and proinflammatory cytokines.

Groups	Control	HFrD	HFrD + folate	HFrD + met.	HFrD + chol.
4-HNE (ng\ml)	191.50 ± 9.22	235.20 [a] ± 11.2	203.30 [ab] ± 7.3	225.40 [ac] ± 8.5	205.70 [abd] ± 9.2
SOD (U/ml)	153.30 ± 5.80	92.50 [a] ± 6.20	140.36 [ab] ± 7.5	145.20 [ab] ± 4.6	135.73 [abd] ± 6.3
GSH (ng/ml)	19.20 ± 1.50	8.35 [a] ± 0.7	17.33 [ab] ± 1.1	19.28 [ab] ± 1.3	15.37 [abd] ± 0.9
TNF-α (pg/ml)	8.75 ± 0.8	24.1 [a] ± 3.5	12.3 [ab] ± 1.3	18.3 [abc] ± 1.7	14.1 [abd] ± 2.1
IL-6 (pg/ml)	5.2 ± 0.08	13.1 [a] ± 0.3	5.93 [b] ± 0.1	7.3 [abc] ± 2.8	6.9 [abc] ± 1.8
IL-1ß (pg/ml)	7.55 ± 0.7	16.95 [a] ± 1.1	11.50 [ab] ± 0.9	12.33 [ab] ± 2.1	10.05 [abd] ± 1.3

Values are expressed as means ± SE. (n = 12); a = significance against control; b = significance against HFrD group; c = significance against HFrD + folate group; d = significance against HFrD + met. at P ≤ 0.01.

levels of HDL-c and hepatic total PL. Whereas, rats fed on folate-, choline-, and methionine-supplemented diets showed a considerable reduction (P ≤ 0.01) in TL, TG, TC, and LDL-c levels. Results demonstrated that the folate-supplemented group (HFrD+ folate) exhibited the greatest advancement in lipids biomarker values, followed by the choline and methionine-added groups, respectively, when compared to the HFrD group. HFrD-induced liver function disturbance was denoted as a significant reduction (P ≤ 0.01) in the serum values of ALB, significant increases in serum TBA levels, and activities of ALT and AST enzymes compared with the control group. There was an improvement observed in serum ALT and AST activities and ALB and TBA levels in rats that had been fed on diets supplemented with folate (HFrD+ folate) and choline (HFrD+ choline) compared with the control group. Serum levels of FFA, FAS, and ACC activities were increased in the HFrD rats compared with the control rats. An improvement was observed in the folate-treated rats (HFrD + folate) compared to the HFrD rats. No significant differences in serum FAS and ACC activities were found between rats supplemented with methionine or choline. (Table 2).

The results in Table 3 showed that HFrD-prompted oxidative stress and was represented as a substantial (P ≤ 0.01) decrease in SOD and GSH levels and an elevation in 4-HNE level when compared with the control rats. Alternatively, feeding the rat with folate (G3), methionine (G4), or choline (G5), improved the levels of oxidative stress biomarkers. The L-methionine-supplemented group, followed by the folate- and choline-supplemented groups, experienced the greatest improvements in oxidative stress biomarker values. Moreover, the results illustrated that the values of TNF-α, IL-6, and IL-1β in rats fed diets supplemented with folate, methionine, and choline were

substantially upregulated when compared with the control rats.

Discussion

NAFLD pathogenesis has been linked to altered OCM. The current study results verified that feeding rats high-fructose diets resulted in considerable changes in the hepatic OCM pathway, which significantly increased serum TC, FFA, FBG, hepatic TL, hepatic TG, and LDL. The findings of the current work have shown a strong correlation between methyl-donor and lipid metabolism. The results displayed that the folate-supplemented rats (HFrD+ folate) exhibited the greatest improvement in hepatic TL, hepatic TG, hepatic PL, serum TC, LDL-c, FFA, and FBG levels and enzyme activities of FAS and ACC, followed by choline- and methionine-supplemented diets. Previous studies have demonstrated that folic acid supplementation had a positive influence on hepatic TG levels and attenuated the inflammatory response in mice fed a high-fat diet [8]. Additionally, studies have revealed that folic acid, through the hepatic activated protein kinase pathway, may lessen abnormal lipid metabolism and cholesterol deposition in the liver [9]. Significantly reduced FBG values in the folate group suggested that folic acid might be implicated in the management of glucose metabolism in disorders like NAFLD. OCM and NAFLD may be linked by a number of processes, but it appears that the production of very low-density lipoprotein (VLDL) and liver lipid export are the most significant.

Choline is used in transporting TG in VLDL, and abnormally high levels of one-carbon metabolites may decrease the secretion of VLDL, which then causes fat to build up in the liver. Since phosphatidylcholine is necessary for the formation of the VLDL envelope, its absence prevents the liver from exporting triglycerides, which causes them to build up in the cytosol. Phosphatidylcholine is formed in the liver by methylation of phosphatidyl-ethanolamine or from integration of preformed choline from food [10]. It has been suggested that choline supplementation had no protective impact on fatty liver in male mice fed a choline supplemented high-fat diet at a dose of 1.3 g/kg. However, they maintain mice blood cholesterol values within the normal range, thus improving their ability to function and preventing harmful liver changes [11]. Studies have also demonstrated that choline's three labile methyl groups can be released and used to treat steatosis in mice [12] and via direct combination with phospholipid through the cytidine 5'-diphospho–choline pathway [13].

Methionine and folate pathways are connected in OCM. Methionine content is influenced by dietary intake, protein breakdown, and the remethylation of homocysteine (Hcy). Several processes, such as remethylation by methionine synthase, can convert Hcy back into methionine, which is constrained by 5-methyltetrahydrofolate's (methyl-THF) availability or betaine synthesized from choline. S-adenosylmethionine (SAM) can then be produced from methionine, which is used in the majority of methyltransferase reactions as a universal methyl donor, such as the synthesis of phospholipids and methylation of proteins and nucleic acids [14]. Then, SAM is changed into S-adenosyl-L-homocysteine (SAH), and finally into Hcy. This Hcy remethylation process connects the methionine cycle to the folate cycle. Methyl-THF is produced from folate that is absorbed from dietary food, the predominant form of folate found in plasma. Then, folic acid supplement enters the cycle by dihydrofolate (DHF). In these pathways, the main function of folate is supplying or receiving one-carbon units. However, THF is the main acceptor molecule that is transformed into 5,10-methylene tetrahydro-folate (methylene-THF) and then by the methylene-THF reductase enzyme, resulting in the formation of methyl-THF. The methyl group methyl-THF is then used in the Hcy remethylation reaction [15].

Oxidative stress is the primary consequence of the abnormal functioning of one-carbon and lipid metabolic pathways. The results indicated that HFrD induced inflammation and oxidative stress denoted as a significant decrease in serum values of oxidative stress markers (GSH and SOD) and a significant increase in 4-HNE and proinflammatory cytokines values (TNF-α, IL-6, and IL-1β). As a methionine metabolite, GSH is the most abundant non-protein sulfhydryl group in cells, which is a protective agent against oxidative stress. In addition to GSH and SOD, 4-HNE is a well-known inducer of oxidative stress and one of the main end products of lipid peroxidation. It also plays a significant role in scavenging ROS in mammals [16].

Various studies postulated that fructose consumption boosts free radical formation and inflammation response [17]. It is proposed that fructose supports the transition from NAFLD to NASH. In mild inflammation, lipids promote inflammatory liver by the formation of unusual cytokines such as IL-6, TNF-α, and IL-1 β. Cytokine formation prevents insulin signaling in hepatocytes, resulting in hyperglycemia. A key, and early, complication is the induction of hepatic VLDL synthesis or increased free fatty acids flow from adipose tissue into the liver [18]. Oxidative stress is initiated because of the excessive production of reactive oxidants and protecting antioxidants, causing interrupted redox signaling, which ultimately results in DNA damage and molecular tissue damage. Numerous liver conditions, including NAFLD, are characterized by oxidative stress, in which free radicals damage proteins, lipids, and cell membranes, leading to tissue damage [19]. Previous animal models of NAFLD have revealed a relationship between the extent of steatosis and lipid peroxidation in the liver [20]. It has been proven that reactive oxygen species have an impact on numerous processes leading to hepatic fibrosis. A number of correlated pro-oxidative mechanisms function together, including mitochondrial dysfunction. Increased FFA metabolism and lipotoxicity may be the cause of diminished mitochondrial function [19]. The influx of FFAs and accumulation of TG in the hepatocytes are the primary mechanisms by which NAFLD develops.

Moreover, the findings of this study demonstrated that HFrD-induced liver function disturbance was represented as a significant reduction in serum levels of ALB and significant increases in serum TBA levels and activities of ALT and AST enzymes. Recovery was observed in serum values of ALT and AST activities, ALB, and TBA in rats that were fed on diets supplemented with folate, methionine, and choline. AST and ALT are formed mainly in the liver and increased levels of these enzymes in the serum are directly related to liver damage progression. Combinations of serum proteins and metabolites are also used to determine the severity of certain liver

alterations, for instance, TBA or bilirubin [21].

Enzymes like FAS, one of the important metabolic enzymes for lipogenesis, may contribute to the promotion of NAFLD. In this pathway, acetyl CoA is transformed into malonyl CoA by ACC, and this molecule is then transformed into long-chain saturated fatty acids by FAS. Nutritional supplements containing folate, choline, and methionine have been demonstrated to slow the enhancement of NAFLD. The current study's findings demonstrated that the levels of GSH, SOD, 4-HNE, TNF-α, IL-6, and IL-1β in rats fed on diets supplemented with folate, methionine, and choline were significantly upregulated. The values of serum inflammatory biomarkers partly displayed the inflammatory response during disease progression. The methionine and folate-supplemented rats had the most significant improvement in oxidative stress and inflammatory biomarkers levels followed by the choline-supplemented groups. Elevated levels of antioxidant biomarkers following folic acid, methionine, and choline supplementation may suggest an increase in antioxidant defenses against free radical damage. In this research, supplemental folic acid altered oxidative status markers by reducing oxidative stress. Recent studies have suggested that folate may have preventive effects through free-radical scavenging activity. However, folic acid supplementation had positive effects on hepatic TG levels and reduced the inflammatory stimulus in mice fed a high-fat diet [22]. Previous research has shown that dietary choline might modify immune responses by upregulating mRNA expression of the anti-inflammatory cytokine IL-6 and downregulating pro-inflammatory biomarker synthesis in vertebrates [23].

In conclusion, methyl-donor supplementation can prevent the advancement of lipid accumulation in the liver tissues of animals with NAFLD. Methyl-donor supplementation resulted in major changes in OCM metabolites and decreased hepatic free fatty acid values. These effects are associated with an increased FAS and ACC activity that links C1-metabolism and β-oxidation. In conclusion, our research has shown that supplementing with methyl donors can prevent the progression of lipid accumulation in liver tissues with NAFLD.

Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and human rights statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. No animal or human studies were carried out by the authors for this article.

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Conflict of interest

The authors declare no conflict of interest.

References

1. Leoni S, Tovoli F, Napoli L, Serio I, Ferri S, Bolondi L. Current guidelines for the management of non-alcoholic fatty liver disease: A systematic review with comparative analysis. World J Gastroenterol. 2018; 24(30):3361.

2. Zeng JD, Wu WK, Wang HY, Li XX. Serine and one-carbon metabolism, a bridge that links mTOR signaling and DNA methylation in cancer. Pharmacol Res. 2019; 149:104352.

3. Xin FZ, Zhao ZH, Zhang RN, Pan Q, Gong ZZ, Sun C, et al. Folic acid attenuates high-fat diet-induced steatohepatitis via deacetylase SIRT1-dependent restoration of PPARa. World J Gastroenterol. 2020; 26(18):2203-2.

4. Guerrero L, Paradela A, Corrales FJ. Targeted proteomics for monitoring one-carbon metabolism in liver diseases. Metabolites. 2022; 12(9):779.

5. Ouyang Y, Wu Q, Li J, Sun S, Sun S. S-adenosylmethionine: a metabolite critical to the regulation of autophagy. Cell Prolif. 2020; 53(11):e12891.

6. Li Z, Wang F, Liang B, Su Y, Sun S, Xia S, et al. Methionine metabolism in chronic liver diseases: an update on molecular mechanism and therapeutic implication. Signal Transduct Target Ther. 2020; 5(1):280.

7. Bakir MB, Salama MA, Refaat R, Ali MA, Khalifa EA, Kamel MA. Evaluating the therapeutic potential of one-carbon donors in nonalcoholic fatty liver disease. Eur J Pharmacol. 2019; 847:72-82.

8. Liu Y, Shen J, Yang X, Sun Q, Yang X. Folic acid reduced triglycerides deposition in primary chicken hepatocytes. J Agric Food Chem. 2018; 66(50):13162-72.

9. Sid V, Wu N, Sarna LK, Siow YL, House JD, O K. Folic acid supplementation during high-fat diet feeding restores AMPK activation via an AMP-LKB1-dependent mechanism. Am J Physiol Regul Integr Comp Physiol. 2015; 309(10):R1215-R25.

10. Mato JM, Alonso C, Noureddin, M, Lu SC, Biomarkers and subtypes of deranged lipid metabolism in non-alcoholic fatty liver disease. World J Gastroenterol. 2019; 25(24):3009.

11. Al Rajabi A, Castro GS, da Silva RP, Nelson RC, Thiesen A, Vannucchi H, et al. Choline supplementation protects against liver damage by normalizing cholesterol metabolism in Pemt/Ldlr knockout mice fed a high-fat diet. J Nutr. 2014; 144(3):252-7.

12. Brosnan ME, Brosnan JT. Formate: the neglected member of one-carbon metabolism. Annu Rev Nutr. 2016; 36:369-88.

13. Walker AK. 1-Carbon cycle metabolites methylate their way to fatty liver. Trends Endocrinol Metab. 2017; 28(1):63-72.

14. Wu S, Zhang J, Li F, Du W, Zhou X, Wan M, et al. One-carbon metabolism links nutrition intake to embryonic development via epigenetic mechanisms. Stem Cells Int. 2019; 2019:3894101.

15. Guéant JL, Oussalah A, Zgheib R, Siblini Y, Hsu SB, Namour F. Genetic, epigenetic and genomic mechanisms of methionine dependency of cancer and tumor-initiating cells: What could we learn from folate and methionine cycles. Biochimie. 2020; 173:123-8.

16. Li W, Tang R, Ma F, Ouyang S, Liu Z, Wu J. Oxidative stress and 4-hydroxy-2-nonenal (4-HNE): Implications in the pathogenesis and treatment of aging-related diseases. J Immunol Res. 2022; 2022:2233906. DOI: 10.1155/2022/2233906

17. Tappy L. Fructose-containing caloric sweeteners as a cause of obesity and metabolic disorders. J Exp Biol. 2018; 221(Pt Suppl. 1):jeb164202. DOI: 10.1242/jeb.164202.

18. Hirano T. Pathophysiology of diabetic dyslipidemia. JAT. 2018; 25(9):771-82.

19. Bovi APD, Marciano F, Mandato C, Siano MA, Savoia M, Vajro P. Oxidative stress in non-alcoholic fatty liver disease. An updated mini review. Front Med (Lausanne). 2021; 8:595371. DOI: 10.3389/fmed.2021.595371.

20. Lau JK, Zhang X, Yu J. Animal models of non-alcoholic fatty liver disease: current perspectives and recent advances. J Pathol. 2017; 241(1):36-44.

21. Li J, Luu LDW, Wang X, Cui X, Huang X, Fu J, et al. Metabolomic analysis reveals potential biomarkers and the underlying pathogenesis involved in Mycoplasma pneumoniae pneumonia. Emerg Microbes Infect. 2022; 11(1):593-605.

22. Sid V, Shang Y, Siow YL, Hewage SM, House JD, O K. Folic acid supplementation attenuates chronic hepatic inflammation in high-fat diet fed mice. Lipids. 2018; 53(7):709-16.

23. Zhao HF, Jiang WD, Liu Y, Jiang J, Wu P, Kuang SY, et al. Dietary choline regulates antibacterial activity, inflammatory response and barrier function in the gills of grass carp (Ctenopharyngodon idella). Fish Shellfish Immunol. 2016; 52:139-50.

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